Published in final edited form as:

Ann Neurol. 2014 June ; 75(6): 972–973. doi:10.1002/ana.24175.

Reactive oxygen species and NLRP3 inflammasome activation (Reply)

Qingyi Ma, PhD^{#1}, Sheng Chen, MD^{#1,2}, Qin Hu, MD, PhD¹, Hua Feng, MD³, John H. Zhang, MD, PhD^{1,4,5}, and Jiping Tang, MD¹

¹Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, California, USA

²Department of Neurosurgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Zhejiang, China

³Department of Neurosurgery, Southwest Hospital, Third Military Medical University, Chongqing, China

⁴Department of Anesthesiology, Loma Linda University, Loma Linda, California, USA

⁵Department of Neurosurgery, Loma Linda University, Loma Linda, California, USA

[#] These authors contributed equally to this work.

We thank Li et al. for their interests in and comments on our published study¹, which demonstrated that mitochondrial reactive oxygen species (mito-ROS) trigger NLRP3 inflammasome activation following intracerebral hemorrhage (ICH). In the letter by Li et al., two concerns were raised: first, mito-ROS should be directly detected; and second, the causative mechanism underlying NLRP3 activation by mito-ROS should be explored.

Regarding the first concern, we agree with Li et al. that mito-ROS detection would be helpful in supporting our hypothesis that mito-ROS, which are governed by mitochondrial permeability transition pores (mPTPs), trigger the NLRP3 inflammasome to facilitate caspase-1 activation and IL-1 β maturation after ICH. To date, two methods have been used to measure mito-ROS in animal tissues and cell cultures. The first involves a system, introduced by Starkov, in which mito-ROS is evaluated in animals using isolated mitochondria in the presence of respiratory chain substrates or inhibitors². This system can reflect the dysfunctions of mitochondria, but cannot be used to detect *in vivo* mito-ROS that results from hemorrhagic stroke-induced brain injury. In the second method, Martin and colleagues injected a fixable, cell-permeable, mitochondria-selective probe (MitoTracker Red CM-H2XRos, Invitrogen) into mouse occipital cortex to track neuronal mito-ROS. The brain slices, stained with a probe dye, were visualized via fluorescence microscopy, as described in their paper published in the *Journal of Neuroscience*³. The fluorescent probe dye method has been widely used to trace mito-ROS in cell culture studies; however, the

Correspondence to: Jiping Tang, M.D. Department of Physiology and Pharmacology, Loma Linda University School of Medicine, Loma Linda, CA 92354, USA Tel: 909-558-4723; Fax: 909-558-0119; jtang@llu.edu.

Potential Conflicts of Interest Nothing to report.

application of this method in animal tissues is limited, because it produces very strong background and thus is hard to quantify. It can be seen, therefore, that these methods are not feasible for *in vivo* experiments, and produce inaccurate measurements of mito-ROS in brain tissue. In our study, we actually attempted to trace the mito-ROS in the brain after ICH with injection of MitoTracker Red CM-H2XROS (Invitrogen) into the mouse brain, but we could not accurately measure mito-ROS due to the strong background. Therefore, we administered Mito-TEMPO (Enzo Life Science), a mitochondria-targeted antioxidant that has superoxide and alkyl radical scavenging properties *via* acting on the mitochondrial matrix⁴, and observed a reduction in total brain ROS in both naïve animals with mito-ROS induction and animals with ICH. We indirectly demonstrated, therefore, the potential involvement of mito-ROS in the initiation of brain inflammation after ICH.

On the second issue raised, regarding how mito-ROS activate the inflammasome, a study published in *Nature* by Zhou and colleagues has demonstrated that mito-ROS promotes NLRP3 inflammasome formation by recruitment of inflammasome components to mitochondria-associated ER membranes (MAMs)⁵. From the same group, another study published in *Nature Immunology* showed that ROS activates the NLRP3 inflammasome *via* release of the ROS-sensitive NLRP3 ligand, thioredoxin-interacting protein, from its inhibitor, thioredoxin⁶. We did not repeat these experiments in our study because our primary focus was to determine how the initial inflammatory response was triggered after hemorrhagic stroke, as shown in an ICH animal model. Our results indicate that a new therapeutic strategy for ICH may be established by targeting NLRP3 inflammasome. However, we agree with Li et al. that future studies are needed to explore the exact roles and mechanisms of ROS-induced NLRP3 inflammasome activation following hemorrhagic stroke.

Acknowledgments

This letter was supported by the NIH, NINDS (NS060936, J.T.; NS053407, J.H.Z.) and The National Basic Research Program of China (973), grant number: 2014CB541600 to H.F.

Reference

1. Ma Q, Chen S, Hu Q, et al. NLRP3 inflammasome contributes to inflammation after intracerebral hemorrhage. *Ann Neurol*. 2014; 75:209–219. [PubMed: 24273204]
2. Starkov AA. Measurement of mitochondrial ROS production. *Methods Mol Biol*. 2010; 648:245–255. [PubMed: 20700717]
3. Martin LJ, Adams NA, Pan Y, et al. The mitochondrial permeability transition pore regulates nitric oxide-mediated apoptosis of neurons induced by target deprivation. *J Neurosci*. 2011; 31:359–370. [PubMed: 21209222]
4. Liu M, Liu H, Dudley SC Jr. Reactive oxygen species originating from mitochondria regulate the cardiac sodium channel. *Circ Res*. 2010; 107:967–974. [PubMed: 20724705]
5. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. *Nature*. 2011; 469:221–225. [PubMed: 21124315]
6. Zhou R, Tardivel A, Thorens B, et al. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. *Nat Immunol*. 2010; 11:136–140. [PubMed: 20023662]